510(k) SUBSTANTIAL EQUIVALENCE DETERMINATION
DECISION SUMMARY

A. 510(k) Number:
k040200

B. Analyte:
Anti-centromere antibody

C. Type of Test:
Semi-quantitative, ELISA

D. Applicant:
RhiGene, Inc
.

E. Proprietary and Established Names:
MESACUP-2 Test CENP-B

F. Regulatory Information:

1. Regulation section:
   21 CFR §866.5100 Anti-nuclear Antibody Immunological Test System

2. Classification:
   Class II

3. Product Code:
   LJM (Anti-nuclear antibody, (enzyme-labeled), antigen, control)

4. Panel:
   IM 82

G. Intended Use:

1. Intended use(s):
   The MESACUP-2 Test CENP-B is a semi-quantitative enzyme-linked immunosorbent assay (ELISA) for the detection of anti-centromere (CENP-B) antibodies in human serum.

   The MESACUP-2 Test CENP-B is intended to be used by clinical (hospital and reference) laboratory personnel.

2. Indication(s) for use:
   The MESACUP-2 Test CENP-B is indicated as an aid in the diagnosis of CREST Syndrome (i.e., calcinosis, Raynaud's phenomenon, esophageal immotility, sclerodactyly, and talangiectasia).

3. Special condition for use statement(s):
   The device is for prescription use only.

4. Special instrument Requirements:
   None

H. Device Description:

The device is an enzyme-linked immunosorbent assay (ELISA) using microtiter plates as the solid phase. The plate wells are coated with recombinant CENP-B antigen, which captures CENP-B autoantibodies present in the patient serum sample. The conjugate is polyclonal goat anti-human IgG, IgM and IgA (heavy chain specific) horseradish peroxidase (HRP) which uses 3,3'5,5' tetramethylbenzidine dihydrochloride/hydrogen peroxide (TMB/H_2O_2) as substrate. The kit contains 2 levels of calibrators (0 units/mL and 100 u/mL) for interpretation of results. A positive and a negative control are included with the kit. The kit also contains sample diluent, wash buffer concentrate and stop solution.

I. Substantial Equivalence Information:

1. Predicate device name(s):
   Quanta Lite Centromere ELISA from INOVA Diagnostics

2. Predicate K number(s):
   k003959

3. Comparison with predicate:

Similarities		
Item	**MESACUP-2 Test CENP-B**	**Predicate**
Indications for Use	For detection of anti-centromere antibodies as an aid in the diagnosis of CREST and related connective tissue diseases.	Same
Assay principle	Indirect ELISA	Same
Sample matrix	Serum	Same
Substrate	TMB	Same
Differences		
Item	**MESACUP-2 Test CENP-B**	**Predicate**
Analyte	anti-CENP-B autoantibodies	Anti-CENP-A and anti-CENP-B autoantibodies
Cut-off	16 U/mL	20 Units
Detection range	5-300 U/mL	0-6 Units
Assay time	150 minutes at Room Temp	90 minutes at Room Temp
Conjugate	HRP-goat anti-human IgG/IgM/IgA	HRP-goat anti-human IgG

J. Standard/Guidance Document Referenced (if applicable):
Not applicable

K. Test Principle:
The assay involves enzyme-linked immunosorbent assay (ELISA) technology. Calibrators and patient sera are incubated with CENP-B antigens for a specified time and then washed. This is followed by incubation with horseradish peroxidase conjugated anti-human IgG, IgA, and IgM. The reaction is then stopped and the color is allowed to develop and measured photometrically.

L. Performance Characteristics (if/when applicable):

1. Analytical performance:

a. Precision/Reproducibility:

Three lots of the MESACUP-2 Test CENP-B were used to determine the intra-assay, inter-assay and inter-lot value precision for the test.

Intra-assay

Intra-assay precision (%CV) was determined by running 3 serum samples (i.e., negative, moderate and high positive) 8 times (i.e., 8 replicates per plate) on 3 separate assays. Three separate plates were randomly selected from each plate-coating run (kit-lot). The mean intra-assay precision for the 3 samples tested on 3 plates from each lot was 3.8% (Range: 2.4-5.4%).

Inter-assay, intra-lot

To determine the amount of variability between plates of the same lot, 3 samples in duplicate were tested on 6 separate assays. Six randomly selected plates randomly selected from the same plate lot were used for each one of 3 separate plate lots. The mean %CV for inter-assay, intra-lot precision was 4.5% with a range of 2.3-8.6%.

Inter-assay, inter-lot

The precision between lots was determined by comparing the values recovered for 3 different samples on 3 different pilot lots. Each of the 3 samples was tested in duplicate and by 2 operators in each assay. The mean inter-assay, inter-lot %CV was 2.3%.

b. Linearity/assay reportable range:

The reportable range of 5-300 U/mL was demonstrated by recovery studies.

c. Traceability (controls, calibrators, or method):

An international reference material for anti-CENP-B antibodies is not available. The assay is calibrated in relative arbitrary units based

on the upper-optical density detection limit using samples that were positive by both ELISA and Double Immunodiffusion (DID).

d. Detection limit:
Not applicable.

e. Analytical specificity:
Several substances were added to three patient specimens (i.e., negative, moderate, and high) each to test for interference. Based on the results summarized below, the addition of these substances at the levels tested did not affect the assay results.

Substance	Level Range (U/mL)	Low			Moderate			High		
		Mean (U/mL)	*SD*	*%CV*	*Mean (U/mL)*	*SD*	*%CV*	*Mean (U/mL)*	*SD*	*%CV*
Hemoglobin	0 – 480	53.9	2.06	3.8	84.8	4.17	4.9	191.8	4.79	2.5
Bilirubin C	0 – 20.4	55.1	4.3	7.8	82.3	5.68	6.9	192.2	7.89	4.1
Bilirubin F	0 – 18.7	48.9	3.54	7.2	74.7	1.97	2.6	191.8	2.99	1.6
Chyle	0 – 2780	53.8	3.52	6.5	83.1	4.26	5.1	191.7	2.72	1.4
Rheumatoid Factor	0 - 520	64.7	1.95	3.0	100.0	2.9	2.9	205.6	3.01	1.5

f. Assay cut-off:
A healthy sample population consisting of 266 unselected serum samples (in duplicate) was tested for anti-CENP-B antibodies with both the MESACUP-2 Test CNEP-B and the DID method. In addition, a population of 691 collagen disease specimens were tested, 5.1% (35/691) of which were positive for ACA antibodies by DID. The cut-off was established by comparing the frequency distribution of values obtained for both populations with the MESACUP-2 Test CENP-B to the DID results for positive and negative agreement. Based on this comparison, the best overall accuracy compared to DID was established at 16 U/mL. Therefore, this was selected as the cut-off. Note: All 35 DID positive collagen specimens were also positive according to the MESACUP-2 Test CENP-2 results with this cut-off. There is no equivocal (gray) zone for this assay.

2. Comparison studies:

a. Method comparison with predicate device:
The tables below shows the results of comparison of serum samples (N=80) that were tested with the MESACUP-2 Test CENP-B and the predicate device.

	Quanta Lite +	**Quanta-Lite --**
MESACUP-2 +	**18**	**1**
MESACUP-2 -	**3**	**58**

STATISTIC	Value	95% CI
Prevalence	0.2625	0.1733 - 0.3648
Positive agreement	0.9474	0.7189 - 0.9972
Negative agreement	0.9508	0.8540 - 09872
Total agreement	0.9490	0.7864 - 0.9922

b. Matrix comparison:
Serum is the only recommended matrix.

3. Clinical studies:

a. Clinical sensitivity:
Clinical sensitivity for the MESACUP-2 Test CENP- was determined by testing a population of CREST Syndrome patient serum specimens (n=20). Using the cut-off of 16 U/mL, 90% (18/20) of the samples were positive for anti-CENP-B antibodies. The mean value for the CREST Syndrome samples was 79.1 U/mL. Single Factor ANOVA analysis that compared this value to the mean for the healthy controls gives a p-value of 2.95×10^{-74} , Therefore, at a level of $p<0.05$ for statistical significance, the results of this population were determined to be statistically different compared to the healthy controls.

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b. Clinical specificity:
The applicant evaluated 266 samples, in duplicate, from 168 consecutive healthy blood donors and used these samples as the normal population. The mean value was 3.0 U/mL (SD = $\pm$ 1.9). Only 2 samples tested positive (0.75%) in this sample population. Therefore, the specificity was 99%.

Similar studies were performed with samples from patients from various autoimmune diseases. The table below provides a summary of the results obtained in these subgroups and in samples from patients with CREST Syndrome.

Disease or Disease Status	N	Mean (U/mL)	SD	Specificity (%)
CREST Syndrome	31	104.8	35.3	97
Rheumatoid Arthritis	47	4.0	8.8	4
Sjögren's Syndrome	47	12.8	36.3	9
Systemic Lupus Erythematosus	103	7.3	18.3	7
Systemic Sclerosis	23	3.0	1.6	0
Mixed Connective Tissue Disease	59	7.6	20.2	7
Polymyositis	9	5.3	3.9	10.3
Dermatomyositis	17	25.3	51.8	24

The minimal cross-reactivity does not seem to be clinically significant, and it is expected to be due to *"cross-over of the various*

autoimmune diseases" (Applicant's conclusion, p. 51). Results similar to those seen here for Dermatomyositis have been reported in the literature where a positive test result for ACA was seen in patients with Raynaud's disease. The data support the specificity of the test for the intended indication

c. Other clinical supportive data (when a and b are not applicable): Not applicable.

4. Clinical cut-off:
   See assay cut-off.

5. Expected values/Reference range:
   The expected value in the normal population is negative.

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M. Conclusion:

The purpose of this 510(k) was to seek clearance to market the MESACUP-2 Test CENP-B ELISA for the detection of anti-CENP-B antibodies in human serum. The data submitted in this 510(k) substantiate a good level of agreement between the results obtained with the MESACUP-2 Test CENP-B and the predicate device, for those conditions specified in the Indications for Use statement. The applicant provided evidence that the devices are substantially equivalent.